NUTRITION, FOOD SAFETY, AND HEALTH

The health of the U.S. citizen depends on the quality and quantity of the country's food supply and the nutrients consumed by individuals. Research is supported which will contribute to the improvement of human nutritional status by increasing our understanding of requirements of nutrients and factors affecting optimal human nutrition. Grants are also awarded in the area of food safety, particularly emphasizing the detection, prevention and control of food-borne disease-causing microorganisms, naturally occurring toxicants and drug residues.

IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH

Panel Manager -Dr. David K.Y. Lei, University of Arizona
Program Director - Dr. Kathleen Ellwood

This program area places an emphasis on research which will increase our knowledge of human nutrient requirements and the factors which influence them. Areas of research which will be emphasized include: nutrient bioavailability, the interrelationship of nutrients, nutrient requirements of healthy individuals across all age groups, mechanisms underlying the relationship between diet and health maintenance (such as the effect of nutrients on the immune system) and cellular and molecular mechanisms underlying nutrient requirements, including nutrient effects on gene expression. In addition, this program encompasses the area of food consumer behavior, including identifying and developing methods to overcome obstacles to adopting healthful food habits, to convey knowledge to target audiences, and to ascertain factors that affect food choice. Innovative approaches to these research problems are encouraged.

9700751 Regulation of T-helper Cell Function by Dietary Vitamin A
Stephensen, C.B.; Bucy, R.P. **Grant 97-35200-4229**

University of Alabama, Birmingham
Department of International Health **$135,000**
Birmingham, AL 35294-0008 **2 Years**

Our overall project goal is to determine how different dietary vitamin A levels affect the normal development of the immune system. Vitamin A deficiency transiently increases the production of cytokines (in particular, -interferon) which enhance the development of type 1 T-helper (Th1) cells. This suggests that vitamin A deficiency can permanently alter the character of an immune response to infection or immunization, enhancing the development of Th1-mediated immunity and thus impairing development of Th2-mediated immunity. Furthermore, we have found that high dietary levels of vitamin A may have the opposite effect on the immune response. This alteration of the pattern of the immune response by vitamin A has important implications. Th1 responses generally induced cell-mediated immunity in response to intracellular pathogens (such as viruses) and Th2 responses evolve in response to extracellular pathogens where the antibody response is of greater importance. Factors which affect the Th1/Th2 balance of an immune response thus can affect the outcome of the response (i.e., development of cell-mediated immunity vs. antibody). Our specific research objectives will be to determine if vitamin A regulates the development of Th1 cells toward a Th1 or Th2 phenotype and to determine if this phenotype is permanent or can be subsequently altered. The Th cell phenotype will be characterized by measuring the percent of cells expressing IFN-γ, IL-2, IL-4, and IL-10 mRNA by in situ hybridization, cytokine protein by immunocytochemistry, and supernatant protein concentrations by ELISA.

9700655 Role of Chromium in Carbohydrate and Lipid Metabolism
Vincent, I.B. **Grant 97-35200-4259**

University of Alabama
Department of Chemistry **$180,000**
Tuscaloosa, AL 35487-0336 **3 Years**

For nearly four decades, the element chromium has been known to be important in the proper metabolism of carbohydrates and lipids. However, the nature of chromium and the role it plays at a molecular level have remained unclear. Recently, a biologically-active material called Low-Molecular-Weight Chromium-Binding Substance (LMWCr) has been isolated in sufficient quantity to allow elucidation of its properties and function. Preliminary studies have shown that LMWCr interacts strongly with the protein insulin receptor such that the amplitude of the signal of the hormone insulin transmitted into a cell is increased sevenfold. It is hypothesized that LMWCr functions in a manner reminiscent of the calcium-binding protein calmodulin. In other words, LMWCr is maintained in cells in its inactive, chromium-free form; in response to a chromium flux (resulting from movement of chromium from the blood to insulin-sensitive cells in response to insulin), LMWCr binds chromium and becomes active, stimulating the activity of insulin receptor. Adult-onset diabetes is related to a condition termed insulin resistance in which the body produces sufficient quantities of insulin, but the signal from insulin is not properly transmitted. Understanding the mechanism by which LMWCr can amplify this signal could result in a new treatment for adult-onset diabetes. Thus, the proposed research is designed to elucidate the mechanism by which LMWCr can amplify insulin's signal transmitted by insulin receptor and to determine the three-dimensional structure of LMWCr.

9700579 Regulation of Apolipoprotein B mRNA Editing Protein Gene Expression by Zinc Status
Wu, Y.J. **Grant 97-35200-4237**

University of Arizona
Department of Nutritional Sciences
Tucson, AZ 85721
$90,000
2 Years

Apolipoprotein (apo) B is a vital component of VLDL and LDL, and elevated level of LDL is associated with cardiovascular disease (CVD). There are two forms of apo B, called apo B-100 and B-48, which reproduced from the same apo B gene by a novel process known as apo B mRNA editing. The editing protein is the catalytic enzyme in this process. Lipoproteins containing B-48 are removed from circulation more rapidly than those containing B-100 and may not contribute to CVD. Similar to humans, hamsters only edit apo B in the small intestine and this could explain why the hamster's lipoprotein profile is closer to that of human as compared to other rodent species which have hepatic editing. Editing levels and editing protein gene expression in the liver have been shown to be regulated by several hormonal and dietary treatments including dietary copper and zinc. This project will examine the influence of zinc status on apo B editing in the hamster intestine and in a human intestinal cell line. The effect of zinc status on the expression of editing protein will also be examined. Data obtained from this project will contribute to the understanding of the regulatory mechanisms of apo B editing and will aid in establishing the molecular basis of how zinc deficiency may contribute to the development of atherosclerosis.

9700903 Neural Mechanisms for Associative Memory During the Recognition Phase of Food Intake
Cummings, S.L. **Grant 97-35200-4289**

University of California, Davis
Department of Anatomy, Physiology and Cell Biology
Davis, CA 95616
$90,000
2 Years

A diet balanced in essential amino acids (EAA) is critical for protein synthesis and growth in all animals. EAA are required by the body in order to make proteins, but must be supplied by the diet because they are not made in sufficient quantities by the body. Animals rapidly become anorexic and will significantly reduce their eating when supplied with a diet deficient in an EAA. Recognition of dietary EAA deficiency is a function of a region of the brain called the anterior piriform cortex. Recognition of diet is mediated by interactions (synapses) between nerve cells which are facilitated by neural chemicals called neurotransmitters, which act at receptors' specialized receiving points on cells. Prominent in this role in the piriform cortex is the N-methyl-D-aspartate (NMDA) receptor for the neurotransmitter glutamate. The NMDA receptor is thought to be particularly important in memory processes. Physiological memory and learning may be reflected as real time changes in numbers of NMDA receptors, the locations of NMDA receptors, or the molecular structures of NMDA receptors.

Our studies suggest that a relationship exists in the anterior piriform cortex between NMDA receptors and neurons which contains the peptide neurotransmitters neuropeptide Y (NPY) and somatostatin (SOM). We have shown that NPY or SOM injected into the piriform cortex significantly alter (depress) the eating of diets deficient in EAA. In the brainstem, NMDA receptors have been shown to stimulate neurons containing NPY, SOM. However, no studies have visualized the relationship of NMDA receptors to feeding related neurons of the cerebral cortex, nor have any studies inquired into the adaptations in cortical neurons during the process of recognizing or learning a diet. The proposed studies will use high power electron microscopy to delineate the synaptic relationships of NMDA-type glutamate receptors to neurons in the anterior piriform cortex which contains NPY and SOM, and will explore the adaptations in these ultrastructural relationships during neural recognition of diets deficient in EAA.

9700947 Neural Responses to Disproportionate Amino Acid Diets: Role of Monoamines
Gietzen, D.W. **Grant 97-35200-4477**

University of California, Davis
Department of Anatomy, Physiology and Cell Biology
Davis, CA 95616-8732
$94,141
3 Years

The long term goals of these studies are to determine the cellular and molecular mechanisms underlying the requirements for essential amino acids, as seen in: 1) recognition, and 2) behavioral rejection of diets containing imbalanced essential amino acid profiles. The anterior piriform cortex (APC) of the brain is believed to house the sensor for this recognition. We have shown that the primary event in the recognition step is a reduced concentration of the most limiting essential amino acid in the APC. The second step of the response, rejection, is an anorectic response to the imbalanced die. Our experimental objectives are: (1) to determine the timing of the two steps in these responses, using feeding behavior and neurochemistry in the APC as the animals are introduced to the diet; and (2) to determine the cellular signals underlying these responses in APC tissue, using a brain slice preparation exposed to experimental amino acid mixtures and agents that allow us to test the neurochemical systems involved. These studies will provide valuable information about the importance of selecting balanced amino acid profiles for

a nutritious diet and contribute to the goal of optimal human health. Also, this work can provide the basis for future treatment of anorexia, as well as intervention in food faddism, counter-productive food selection and inappropriate supplementation.

9700652 The Relationship Between Lead and Iron and Behavioral Development in Infants and Young Children
Pollitt, E. **Grant 97-35200-4230**

University of California, Davis
Department of Pediatrics **$150,000**
Davis, CA 95616 **2 Years**

Iron deficiency anemia and high lead levels are significant public health problems among low-income children in the United States; each has deleterious effects on the behavioral development of infants and young children. The proposed study addresses: (1) whether iron interacts with lead to affect the health of children, and (2) whether iron supplementation improves the physiological and functional well-being of anemic children. There are four groups of subjects: group 1 - children with normal iron and safe lead levels; group b - children with poor iron but safe lead levels; group c - children with normal iron but unsafe lead levels; and group d - children with both poor iron and unsafe lead levels. The first phase of the study will test the hypothesis of whether group d children will score lower on behavioral development tests than those in groups b or c. Group a children are expected to score higher on the tests than subjects in the other three groups.

Phase two of the study will test the second hypothesis, which predicts changes in group outcomes after anemic children (in groups b and d) receive iron supplements for three months. Subjects in groups b and d are expected to show improved iron status and improved scores on the developmental tests after receiving iron. We expect children in group b to score similarly to those in group a, and those in group d to score similarly to those in group c. This study will provide new information on how iron and lead influence the development of young children.

9700665 Characterization of Low Protein Induced Secondary Hyperparathyroidism.
Insogna, K.L.; Kerstetter, J.E. **Grant 97-35200-4420**

Yale University
Department of Internal Medicine **$191,000**
New Haven, CT 06520-8020 **3 Years**

Osteoporosis is an important public health problem in the United States. While the post menopausal decline in estradiol is a major cause of osteoporosis, it does not explain the rise in the age-specific incidence of the disease or its cultural variation. These latter phenomena suggest that environmental factors play a role and one important factor may be dietary protein.

Our studies have established dietary protein as an important regulator of calcium metabolism in women. We have found that in healthy women, a low protein, otherwise nutritionally complete diet, induces dramatic changes in calcium metabolism resulting in secondary hyperparathyroidism. Secondary hyperparathyroidism is an appropriate rise in circulating parathyroid hormone in response to a decline in blood calcium. Our studies indicate that the secondary hyperparathyroidism may be due to changes in the way the intestine and the skeleton metabolize calcium.

These observations raise important questions regarding protein requirements and skeletal health since the low protein diet is only slightly below the RDA for adults. To further explore this relationship this proposal will: 1) Examine sex differences in calcium metabolism in response to dietary protein restriction; 2) Determine the dose-response relationships between dietary protein, bone turnover and the calcitropic hormones in women; and 3) Quantify the acute and chronic changes in calcitropic hormones and markers of bone metabolism to varying levels of dietary protein in women. The overall aim of this proposal is to identify the level of dietary protein that will optimize calcium nutrition and bone health.

9700826 Dietary Status of Older Americans and Factors Associated with Healthful Food Practices
Jensen, H.H.; Oakland, M.J. **Grant 97-35200-4235**

Iowa State University
Center for Agricultural and Rural Development **$ 95,000**
Ames, IA 50011-1070 **2 Years**

The population of older adults in the U.S. is increasing rapidly. Nutritional well-being is closely tied to successful aging, as measured by optimal health, independence and other measures of the quality of life. The goal of the proposed research is to assess the adequacy of diets of older adults, examine the extent to which food practices adhere to dietary recommendations, and identify factors which are associated with good dietary practices. The research uses recently available data from the 1994-96 Continuing Survey of Food Intakes by Individuals and the Diet and Health Knowledge Survey (CSFII/DHKS) to examine dietary intake and the role of age and consumer attitudes and knowledge, among other factors, in predicting good dietary outcomes.

The research uses newly developed statistical methods applied to dietary intake survey data to assess the adequacy of the diet of older adults in the U.S. through the estimation of distributions of usual intake of nutrients, other dietary components, and

numbers of servings from Pyramid Food Guide food groups in order to estimate the population and sub-populations at risk for inadequate dietary intake. Direct and indirect effects of age, income, personal health conditions and other factors affecting consumer attitudes and behavior are modeled and evaluated to determine their relative contribution to achieving a health-promoting diet. These results have direct implication for the design and recommendations related to interventions to improve dietary status of the elderly and to develop a comprehensive strategy for ensuring the health and well-being of older adults.

9700943 New Methods of Assessing Vitamin A Status
Olson, J.A.; Tanumihardjo, S.A.; Barua, A. B. **Grant 97-35200-4290**

Iowa State University
Department of Biochemistry and Biophysics **$161,000**
Ames, IA 50011-3260 **3 Years**

Vitamin A deficiency results in an impaired immune response, depressed growth, abnormal reproduction, blindness, and death. Vitamin A inadequacy is a major public health problem in the world, not only in less industrialized countries but also among socioeconomically disadvantaged women and children in the United States. Few sensitive and specific procedures currently exist for assessing the vitamin A status of individuals. In the past we have developed two such procedures, the modified relative dose response (MRDR) test and the isotope-dilution (ID) method, in which vitamin A that contains 4 "heavy" atoms of hydrogen is used. The effect of infections, which markedly influence other indicators of vitamin A status, on the MRDR test will be determined. We anticipate that the MRDR test will be little affected, thereby making it more useful. We are also exploring the use of vitamin A in the ID test that contains "heavy" atoms of carbon (^{13}C). The use of ^{13}C-vitamin A should greatly improve the sensitivity and utility of this test. Finally, a carbohydrate complex of vitamin A acid, termed RBG, is converted to vitamin A acid 40 times faster in vitamin A-deficient rats than in normal rats. We are exploring the utility of this physiologic reaction as the basis for another test of vitamin A status. These studies should contribute not only to our understanding of the extent of vitamin A inadequacy in the United States and abroad but also to a better definition of vitamin A requirements in health and disease.

9700617 Zinc Nutrition and Vascular Endothelial Integrity
Hennig, B.; Toborek, M.; Boissonneault, G.A. **Grant 97-35200-4231**

University of Kentucky
Department of Nutrition and Food Science **$138,000**
Lexington, KY 40506-0054 **2 Years**

Atherosclerosis or heart disease continues to be the number one cause of death in the United States. The proposed study is part of an ongoing research effort in the area of nutrition and endothelial cell function with regard to atherosclerosis. The endothelial cells, which form the lining of the blood vessels, act as a barrier by selectively allowing blood components into blood vessel walls. Because zinc is required for normal cellular repair processes, and because atherosclerosis is believed to begin with vessel wall injury or dysfunction, depressed zinc status may be involved in either initiation of endothelial cell injury or inadequate vascular tissue repair. Little is known about the requirements and function of zinc in maintaining endothelial cell integrity, especially during stressful conditions, such as the inflammatory response in cardiovascular disease. Our studies have indicated that exposing endothelial cells in culture to certain lipids, namely fatty acids, or to blood molecules, such as inflammatory cytokines, increases endothelial permeability to large molecules such as albumin or cholesterol-rich lipoproteins, a change that may be related to the initiation of atherosclerotic processes. Because of its antioxidant and membrane stabilizing property, we hypothesize that zinc will protect against lipid/cytokine-mediated endothelial cell dysfunction and atherosclerosis. Using a cell culture system which allows a well-defined experimental environment, this research proposes to test mechanisms by which zinc provides protection against endothelial cell dysfunction. Results of the proposed studies should provide further insight into optimal zinc nutrition and requirements for maintenance of vascular endothelial cell function.

9700918 Suppressed Cell Proliferation and Differential Gene Expression by N-3 Fatty Acids
Hwang, D. **Grant 97-35200-4258**

Louisiana State University
Pennington Biomedical Research Center **$157,000**
Baton Rouge, LA 70808-4124 **3 Years**

Many studies in humans suggest that aspirin-like drugs can reduce the incidence of colon cancer. The well-documented action of aspirin-like drugs is inhibiting the enzyme that is responsible for producing hormone-like substances called "prostaglandins." It has been shown that the levels of this enzyme and prostaglandins are much higher in colon tissues than normal tissues. Studies using laboratory animals also showed that inhibiting the enzyme reduces the number of polyps, a

premalignant step in colon cancer development. It has been well-documented that consuming sea foods can suppress that production of prostaglandins in the body. This effect is considered to be due to the type of fat found in fish and other sea foods. Thus, the goal of the proposed studies is to determine whether fish oil can mimic the beneficial effect of aspirin-like drugs in reducing the risk of colon cancer.

The proposed studies could yield important information regarding whether the risk of colon cancer, and perhaps other cancers, can be reduced by consuming diets containing nutrients that suppress the production of the hormone-like substances "prostaglandins".

9700577 Metabolism and Function of Retinoic Acid in Quail Embryogenesis
Zile, M.H. **Grant 97-35200-4239**

Michigan State University
Department of Food Science and Human Nutrition **$210,000**
East Lansing, Ml 48824-1224 **3 Years**

This research addresses the function and metabolism of vitamin A in early avian embryonic development. The studies will use vitamin A-deficient and normal quail embryos as a model system so as to examine the role of vitamin A nutrition in early development. The overall objective of this work is to determine how vitamin A-active forms regulate very early embryonic development during the time period when critical growth-regulators specify cell lineage and provide positional information for the development of future tissues and organs. The early development in the vitamin A-deficient quail embryo is grossly abnormal and leads to early embryonic death. The abnormalities can be prevented by an administration of vitamin A active compounds ("rescue"). The specific objectives of the proposed work are to determine if this "rescue" is by the activation of specific genes or transcription factors such as the nuclear retinoic acid receptors RARs or the retinoid-X receptors RXRs, and if a dysfunction of these genes in vitamin A deficiency is linked to the embryonic abnormalities observed.

9700750 Probiotic Supplements, Prebiotics, and Colon Health
Brady, L.J.; Busta, F.F.; O'Sullivan, D.J. **Grant 97-35200-4236**

University of Minnesota
Department of Food Science and Nutrition **$90,765**
St. Paul, MN 551080-6099 **2 Years**

Probiotics (live bacteria such as found in yogurt) and prebiotics (foods which stimulate beneficial bacteria in the colon) are receiving much attention for their health promoting effects, especially in Japan and Europe, but increasingly in the U.S. also. Little is really known about why probiotics are beneficial to the colon or why prebiotics might stimulate some colon bacteria (beneficial), but not others (potential pathogens). Our project will examine the characteristics of bifidobacteria (a probiotic bacteria) that allow it to successfully colonize the human colon without harm while other types of bacteria are excreted or do cause harm. Our project will examine the way that bifidobacteria get energy for growth. Once we understand the basics of energy production, we will examine how various prebiotics affect the energy production of bifidobacteria. We will use those prebiotic "foods" often recommended to humans for health promotion--complex carbohydrates from wheat and soy, as well as fructo-sugars that are now being promoted as "healthy" for the gut. Our experiments will help us understand the effects of diet on the balance of colon bacteria and what might perturb the balance.

9700907 N-3 Fatty Acids and Interferon-gamma
Fritsche, K.L. **Grant 97-35200-4288**

University of Missouri, Columbia
Department of Animal Sciences **$94,000**
Columbia, MO 65211 **2 Years**

My lab has been focused on studying the effects of fish oil consumption on the immune response of mice in order to better understand why these fats may improve health and well-being of people. We have made several significant and novel observations demonstrating how fish oils affect the immune response in mice infected with bacteria. We hypothesize that a central mechanism for these effects is that specific components found in many fish oils, known as n-3 polyunsaturated fatty acids reduce tissue responsiveness to interferon-gamma, by reducing the number of interferon-gamma receptors on cells. Interferon-gamma is made only in response to trauma or infection and is a very potent pro-inflammatory agent. While we need some interferon-gamma to remain healthy, some human diseases are associated with an excessive production or responsiveness to it (e.g., arthritis, thyroiditis, inflammatory bowel disease). The specific aims of this research proposal are: (1) To characterize the impact of the two major n-3 fatty acids found in fish oils, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the expression of interferon-gamma receptors on mouse immune cells; (2) To determine the effect of dietary fish oil, EPA

and DHA on the production of interferon-gamma in mice. Understanding how these unique fatty acids influence this aspect of the immune system is of vital importance for the definition of a diet that supports optimal health.

9700909 Zinc and Growth Factor Regulation of Cell Division
MacDonald, R.S.; O'Dell, B.L. **Grant 97-35200-1626**

University of Missouri, Columbia
Department of Food Science and Human Nutrition
Columbia, MO 65211
$130,000
2 Years

Understanding the processes of growth and development is essential to formulate strategies for improvement of human health and animal production. There is indisputable evidence that zinc exerts a regulatory function in growth and that part, if not most, of this effect occurs at the level of cell division. However, the mechanism by which zinc regulates cell division remains unknown. Because this process requires growth factors, the impairment of cell division could be explained by failure of growth factor binding and the growth factor-dependent initiation of cell division. Our hypothesis is that the first limiting effect of zinc deprivation relates to the failure of one or more functions that reside in the plasma membrane. Particularly, that zinc is essential for function of one or more of the growth factors that initiate and sustain the cell cycle. This in turn accounts for failure of DNA synthesis and the accompanying biochemical defects. This project will examine the hypothesis that zinc affects the biochemical processes initiated in the plasma membrane which regulate cell division, specifically calcium uptake, using 3T3 cells in culture. Models which identify the biochemical pathology in the membrane will be examined to reverse the pathology in vitro. The project will also include experiments to investigate the role of IGF-I in regulating appetite neuropeptides such as NPY and leptin in the zinc deficient rat.

9700900 Molecular Basis for Selenium Regulation of Glutathione Peroxidase mRNA Level
Weiss, S.L. **Grant 97-35200-4257**

University of Missouri, Columbia
Department of Biochemistry
Columbia, MO 65211-0001
Postdoctoral Fellowship
$90,000
2 Years

Selenium is both essential and toxic. We hypothesize that cells can sense and respond to selenium status because of specific genes which direct the fate of selenium. Glutathione peroxidase (GPX) is a protein which contains selenium, and the messenger RNA (mRNA) for GPX is dramatically reduced in selenium deficiency, perhaps to ensure that selenium is used only for critical roles. Thus, we propose that GPX mRNA level is a parameter which directly indicates intracellular selenium status. For the present research project, specific mutations will be introduced into the DNA which encodes GPX and the gene constructs will then be transferred to mammalian cells in culture. Mutant GPX mRNA levels will be determined in selenium-deficient and selenium-adequate cells. These studies will identify the minimal DNA sequences required for selenium regulation of GPX mRNA. The minimal sequences should also be capable of conferring selenium responsiveness to mRNAs which are not normally affected by selenium. A second approach will focus on shifting the cellular selenium requirement for GPX mRNA level by altering the ability of cells to synthesize selenium-containing proteins. The most recent (1996) WHO/FAO/IAEA report suggests that humans require only 40 µg selenium per day, much less than a typical American consumes. This amount of selenium is needed for approximately two-thirds of maximal GPX activity in plasma. The present research project will further our understanding of selenium requirements at the intracellular level so that convenient parameters such as plasma GPX activity might be used with confidence to determine selenium requirements in humans.

9700611 Correlates of Consumption of Energy-Dense, Nutrient-Poor Foods in the U.S.
Kant, A.K. **Grant 97-35200-4292**

City University of New York, Queens College
Department Of Family, Nutrition, and Exercise Sciences
Flushing, NY 11367
$60,000
2 Years

Healthy diets are believed to be those that provide adequate intake of all nutrients while decreasing the risk of major chronic diseases by reducing the intake of energy and fat. The energy-dense, nutrient-poor foods (so-called junk foods) provide energy, fat, and sugar but relatively little amounts of other nutrients. Federal dietary guidance recommends moderation of these foods. Little is known about the extent of contribution of these foods to the American diet, factors related to their use, and the impact of their use on the quality of diets consumed by Americans. The objectives of this study are to examine: 1) correlates of consumption of energy-dense, nutrient-poor foods by adult Americans; 2) the relation of intake of these foods with nutrient adequacy; and 3) the relation of intake of these foods with measures of body fatness. The study will use nationally representative data from the third National Health and Nutrition Examination Survey, (NHANES III, 1988-1994) to examine these questions. The significance of this research is that it will help in determining whether intake of energy-dense, nutrient-poor foods is a barrier

for complying with dietary recommendations and consuming nutritionally adequate diets. Identifying population subgroups at risk for high consumption of energy-dense, nutrient-poor foods will help in design and targeting of suitable nutrition interventions.

9700690 Regulation of Osteoblast Apoptosis by Vitamin D
Welsh, J.E. **Grant 97-35200-4351**

W. Alton Jones Cell Science Center **$135,000**
Lake Placid, NY 12946 **2 Years**

Bone remodeling reflects a balance between osteoclastic bone resorption and osteoblastic bone formation. Imbalances between these two distinct cell-mediated processes underlie a number of important bone diseases, including osteoporosis. It is now recognized that cell numbers reflect not only the rate at which cells proliferate, but also the rate at which cells undergo cell death by *apoptosis*. Although important regulators of osteoblastic proliferation have been identified, little attention has been directed towards understanding the process of apoptosis in bone cells. 1,25 dihydroxyvitamin D (1,25D), the active form of vitamin D, is anabolic for bone and exerts direct effects on osteoblasts. Our studies will test the hypothesis that 1,25D represents an important physiologic suppressor of osteoblast apoptosis. The overall goal of this proposal is to confirm the anti-apoptotic effect of 1,25D in osteoblasts, and to determine if this effect occurs at a defined time point along the vitamin D - induced differentiation pathway. Our studies will use an established osteoblast cell line and primary cultures of rat osteoblasts, both of which are sensitive to 1,25D and undergo *in vitro* differentiation. We anticipate that these studies will identify a novel role for vitamin D in osteoblast physiology, and could provide a mechanistic understanding of the beneficial effects of vitamin D on post-menopausal and/or age related bone loss.

9700661 Vitamin A-Dependent Regulation and Function of *Hox* Genes in Development
Packer, A.I. **Grant 97-35200-4291**

Columbia University College of Physicians and Surgeons **Postdoctoral Fellowship**
Department of Genetics and Development **$89,163**
New York, NY 10032 **2 Years**

Vitamin A is of fundamental importance in maintaining the normal functions of cells and tissues in fetal development and in adult life. One recent finding suggests that a slightly elevated intake of vitamin A during pregnancy is associated with an increased risk of birth defects; others suggest that vitamin A supplementation may be a promising treatment for premature infants prone to respiratory distress and infection. A better understanding of the roles and mechanisms of action of the vitamin A derivatives--the retinoids--is needed in order to determine appropriate levels of dietary vitamin A during pregnancy and for the identification of other ways in which nutritional status affects human health. Basic studies in experimental models such as the mouse have demonstrated that the retinoids function by regulating the expression of a variety of genes during fetal development. I have proposed a series of experiments to study the retinoid-dependent regulation and potential function of a *Hox* gene, *Hoxa*-4, in mice. *Hox* genes are critically important for normal embryonic development in many organisms, including mice and humans, and a growing body of evidence suggests that altered vitamin A metabolism affects development, at least in part, by altering *Hox* gene expression. Our preliminary observations indicate that *Hoxa*-4 is likely to be regulated directly by retinoic acid (a vitamin A derivative), and its expression in tissues where retinoids are known to be important—the hindbrain, lung, and kidney--makes it an excellent model in which to study how suboptimal and/or elevated levels of retinoids affect development. The study will examine the manner and timing of vitamin A-dependent activation of *Hoxa*-4, and will explore the possibility that vitamin A affects fetal lung and kidney development by regulating the expression of *Hoxa*-4 and other related *Hox* genes.

9700765 Impact of Flavonoid on Redox Regulation of Gene Expression
Loo, G. **Grant 97-35200-4260**

University of North Carolina, Greensboro
Department of Food, Nutrition and Food Service Management **$95,000**
Greensboro, NC 27412-5001 **2 Years**

Diets rich in fruits and vegetables seem to protect against the development of certain chronic diseases such as coronary heart disease (CHD) and cancer, which are the two leading causes of death in this country. It is still unclear what natural substances in fruits and vegetables are the actual health-promoting nutrients. This study will examine the class of antioxidants known as flavonoids. Our general hypothesis is that flavonoid antioxidants can neutralize free radicals during oxidative stress, and inhibit the free radicals from over-activating specific genes. When these genes function at a normal level, they help maintain health and well-being. On the other hand, when these genes function at an abnormally high level due to over-activation of the genes by free radicals, the development of CHD, cancer, and other chronic diseases may be promoted. In order to test the above hypothesis, the relating antioxidant activity of a select group of flavonoids will be initially determined by evaluating their capacity

to directly neutralize a chemical free radical reagent and also to inhibit lipid peroxidation that can be induced by free radicals. Next, those flavonoids with adaptable antioxidant activity will be pre-incubated with some cultured cells to allow for cellular uptake of the flavonoids. The flavonoid-loaded cells will be subjected to oxidative stress to generate free radicals, thus mimicking what might happen inside the body. The extent of free radical-mediated activation of two genes that are implicated in the development of CHD and cancer will then be determined. It is anticipated that introduction of flavonoid antioxidants into the cells will suppress free radical-mediated, over-activation of the genes of interest. Completion of this project should allow us to better understand at the molecular level how a diet rich in fruits and vegetables lower the risk of especially CHD and cancer while enhancing human health statue.

9700620 Effect of Selenium on Selenoproteins in Human Muscle and Brain Cells
Whanger, P.D. **Grant 97-35200-4261**

Oregon State University
Department of Agricultural Chemistry
Corvallis, OR 97331-7301
$203,000
3 Years

Selenium deficiency has been shown to result in both cardiac and muscular disorders in humans. Although there is meager information on this, there is reason to believe that selenium could be important in brain metabolism in humans. It is known with animal studies that selenium is metabolized differently by the brain as compared to other tissues such as the heart and muscle, and the present research should provide some information on brain disorders in humans. A selenium containing protein, which was discovered in my laboratory, has been shown to have a greater affinity for the brain than other tissues in rats. Because of the limitations with humans, this is the reason it is proposed to use tissue cultures to investigate this more thoroughly. Two types of cells from human brain and cells from human muscle will be used to study the effects of selenium deficiency on their metabolism and to investigate the effects of oxidative stress on them. Oxidative stress increases in humans who are under stress as a result of various factors. This oxidative stress can lead to damage to the cell if continued for an extended length of time, and consequently result in health problems to the individual. Molecular biology techniques will be used to increase the selenium containing proteins in these cells to see if they are more resistant to the damages caused by oxidative stress. Likewise, this technique will be used to eliminate selenoproteins from these cells to determine whether these depleted cells will be more sensitive to oxidative stress. A number of methods will be used to assess oxidative stress in these cells.

9700692 Garlic and Nitrosation
Milner, J.A.; Kris-Etherton, P.M.; Jensen, G. **Grant 97-35200-4679**

The Pennsylvania State University
Department of Nutrition
University Park, PA 16802-6504
$129,896
2 Years

Information from several sources suggests diet can significantly influence human health and disease resistance. The proposed studies seek to expand knowledge about the health benefits of garlic and related allium foods. While the intake of garlic, onions and leeks has been implicated in reducing cancer risk, most of the supporting evidence comes from laboratory models. The proposed studies will evaluate in humans the ability of garlic, onions, and leeks to reduce the formation and bioactivation of a broad class of naturally occurring compounds knows as nitrosamines that are recognized as potent teratogenic, mutagenic, and carcinogenic agents. The ability of garlic to suppress the formation of these compounds will be accomplished by monitoring in urine the occurrence of a non-mutagenic and non-carcinogenic compound, N-nitrosoproline. N-nitrosoproline is a known to arise from nitrite formed as a result of normal dietary habits or as a result of normal metabolism. Thus, studies will determine if dietary allium foods can block both exogenous and endogenous formation of nitrosamines using this and other biomarker of nitrosamine formation and metabolism. Special attention will be given to establishing the minimum quantity of garlic and related foods needed to be given to provide protection against nitrosamines. The information gained from these studies will not only impact the understanding of how garlic and related sulfur compounds influence nitrosamines, but should expand the understanding of how specific sulfur compounds in foods can influence general drug metabolism.

9700861 Mechanisms of Endothelial Cell Dysfunction During Selenium Deficiency
Sordillo, L.M.; Reddy, C.C. **Grant 97-35200-4293**

The Pennsylvania State University
Department of Veterinary Science
University Park, PA 16802-3500
$96,000
2 Years

Disruption of endothelial cell function was suggested to be a primary event leading to both acute and chronic disease processes such as atherosclerosis, thrombosis, and peripheral vascular disease. It is well established that overall nutritional status of the host will influence endothelial cell integrity and consequently impact the development of certain pathologic conditions.

Although sele/nium (Se) status is crucial to the development of vascular dysfunction, the exact mechanisms by which this micronutrient is able to influence endothelial cell metabolism is not known. The long term goal of this grant application is to elucidate the mechanisms involved in endothelial cell dysfunction which occurs as a result of inadequate Se nutrition. The central hypothesis to be tested is that Se deficiency can influence the expression of key enzymes associated with eicosanoid biosynthesis and modify the arachidonic acid product profiles in endothelial cells. This altered eicosanoid production is, in part, responsible for the modified expression of critical effector molecules which influence vascular adhesiveness. This hypothesis is based on the premise that Se status can influence the synthesis of prostaglandin and leukotrienes by endothelial cells during oxidant stress through the regulation of intracellular glutathione peroxidase. These eicosanoids are known to profoundly influence leukocyte-endothelial cell interaction. Results from these studies will advance our understanding of the influence of Se nutritional status on essential endothelial cell responses. This work will provide the molecular basis of how Se deficiency can influence resistance to metabolic vascular disorders.

9700684 Zinc Repletion on Cognition and Growth of Mexican American Children
Sandstead, H.H. **Grant 97-35200-4577**

The University of Texas
Preventive Medicine and Community Health **$94,664**
Galveston, TX 77555-1109 **2 Years**

Zinc is essential for cognition, neuromotor function and growth. Research on poor urban Chinese children found that zinc and micronutrient repletion for ten weeks significantly improved neuropsychological performance (cognition and neuromotor function), and growth. Micronutrients alone had little effect on brain function, but did improve growth. Previous findings from Brownsville, TX suggest that usual diets of poor Mexican-American children are low in bio-available zinc and other micronutrients. Therefore this research will determine if repletion with zinc and micronutrients improves performance of poor Mexican-American children from Brownsville, TX, in a manner similar to that observed in Chinese children. The research design will be a ten week, double-blind, randomized, controlled treatment trial. The four treatment groups will be given placebo, 24 mg zinc, micronutrients, or 24 mg zinc with micronutrients. Treatments will be administered by teachers five times per week. (1) Two hundred healthy 6-7 year olds, without serious learning or behavioral problems whose height is $\leq$ 50th percentile of the US standard will be studied. At baseline and follow-up neuropsychological performance (by computerized tasks), (2) knee height (by a highly accurate electronic device), and anthropometry (by standard techniques) will be measured. Background data will include medical, socio-economic and dietary histories, physical examinations, and zinc content of hair and blood plasma. Positive findings will have important implications for dietary guidelines for children. In addition, the findings will indicate the need to determine if low zinc nutriture is one of the factors responsible for poor cognitive performance and short stature of poor children in developing countries.

9700578 Environmental Influences on Children's Diets
Cullen, K.W. **Grant 97-35200-4233**

University of Texas M.D. Anderson Cancer Center **New Investigator Award**
Department of Behavioral Science **$80,000**
Houston, TX 77030-4095 **2 Years**

Inadequate intake of fruit and vegetables and high intake of dietary fat are associated with increased risks for cancer. Children eat fewer fruit and vegetables and more fat than recommended. Research has revealed that personal factors are only low predictors of fruit and vegetable consumption, suggesting that environmental factors may be important in understanding children's food consumption. Little is known about how environmental factors influence children's food consumption. This pilot study will identify how three environmental factors (parent motivation, knowledge, and skills to promote healthy diets in children, food availability/accessibility, and social factors) influence 9-12 year old children's fruit, vegetable, and fat consumption. In the first year, focus groups will be conducted with African-American, Euro-American, and Mexican-American 4th-6th grade children and their parents to investigate dietary practices and develop and test survey questions on environmental influences on food intake. In year 2, another group of African-American, Euro-American, and Mexican-American 4th-6th grade students will complete 7-day food records and the social influences questionnaire. Availability/accessibility of the target foods in the school cafeteria and in fast food and other restaurants commonly identified in the children's food records will be assessed by observation. Parent motivation, knowledge, and skills to promote children's healthy diets and home availability/accessibility of the target foods will be obtained by phone data collection from parents. The environmental factors will be related to consumption and compared across ethnic groups. If environmental factors substantially influence children's diet, interventions will need to target them to be successful.

9700693 The Role of Docosahexaenoic Acid in Infant Development
Jensen, C.L. **Grant 97-35200-4234**

Baylor College of Medicine
Department of Pediatrics
Houston, TX 77030
$100,000
3 Years

A special fat, called docosahexaenoic acid (or DHA), makes up a significant part of the membranes in the cells of the brain and the portion of the eye called the retina. Getting enough DHA may be important in helping babies to develop normally and have good visual function. DHA is present in breast milk, but the amount in breast milk varies depending, in part, on the diet of the breast-feeding mother. Based on studies we have done previously, the amount of DHA is higher in the breast milk of mothers who receive DHA in their diets. It is not known if infants who receive breast milk with more DHA develop better or have better visual function, but this is a distinct possibility which this study will determine. To do this, mothers who have decided to breast-feed their baby for at least four months will be assigned, by chance, to one of two groups -- one group will receive extra DHA every day for the first four months after delivery and the other group will not receive extra DHA. The amount of DHA in each mother's milk will be measured when infants are one, two and four months of age. Tests of each infant's vision will be done at four and eight months of age, and tests of each infant's development will be done at 18 months of age. We will then see if infants who received more DHA in the breast milk perform better on these tests of vision and development.

9700675 Dietary Salt and Bone Turnover in Postmenopausal Women
Massey, L.K. **Grant 97-35200-4240**

Washington State University
Food Science and Human Nutrition
Pullman, WA 99164-6376
$66,000
1 Year

Eating more dietary salt (sodium chloride) increases the rate of loss of calcium from the blood into urine. Unless the body compensates for this loss, bone will be broken down to replace the calcium needed in the blood. Older people appear to be less able to adapt to the challenge of a high salt diet. At greatest risk are women past menopause who are not taking replacement hormones. When bones lose strength because breakdown is faster than repair, fractures of the spine, hip and wrist are more likely. This study will compare the effect of high and low dietary salt consumption on biochemical markers of bone breakdown and repair in postmenopausal women not taking hormones. Ten women will live for two weeks in a metabolic unit and eat a low salt diet. For one of the two weeks, one and one-half teaspoons of salt will be added. Because bone breakdown and repair is affected by physical activity and sleep, bone turnover markers will be measured several times during the seventh day on each diet. Blood calcium and two hormones which regulate calcium levels will also be measured to determine how salt increases urine calcium loss, and how much compensation occurs. The results of this study will be useful in making dietary recommendations to reduce risk of osteoporosis, both in counseling women individually and in determining national food and nutrition policies such as dietary guidelines and food label claims.

9700657 Vitamin B-6 Requirements of Women in Relation to Immune Competence
Shultz, T.D.; Leklem, J.E. **Grant 97-35200-4290**

Washington State University
Department of Food Science & Human Nutrition
Pullman, WA 99164-6376
$94,000
2 Years

The current Recommended Dietary Allowance (1989 RDA) for vitamin B-6 is 1.6 mg/d for adult women. The RDA is based on a limited number of requirement studies conducted with limited numbers of subjects. This study will assess vitamin B-6 requirements of women and determine relationships among B-6 status indices and immune competence. The effects of vitamin B-6 depletion and repletion on B-6 metabolite profiles and immune responses as assayed from plasma, red and white blood cells, and urine will be evaluated in a controlled metabolic setting over two months. This study will determine how controlled diets differing in B-6 may affect various indices of B-6 status and requirements in young women. Plasma, red and white blood cells, and urinary concentrations of individual B-6 metabolites, and total B-6 will be determined. Assessment of immune status will involve measuring various plasma and white blood cell immune parameters. This study will permit a carefully controlled evaluation of whether the RDA for vitamin B-6 is adequate for adult women by relating change in immune responses (functional endpoints) to alterations in B-6 intake and change in tissue levels of B-6 status indicators, thereby allowing an estimation of B-6 requirements based on immune competence. The results of this study will augment the requirement data available to set an accurate RDA for vitamin B-6 in humans. This research will contribute to the long-range goal of improving or enhancing human health by increasing our knowledge of vitamins in food required to satisfy human needs.

9700712 IRPs and the Regulation, of Iron and Energy Metabolism in Iron Deficiency

Eisenstein, R.S. **Grant 97-35200-4232**

University of Wisconsin, Madison
Department of Nutritional Sciences
Madison, WI 53706

$215,000
3 Years

Iron is an essential but potentially toxic nutrient for virtually all organisms. Insufficient intake of iron can impair human and animal health. Iron deficiency ultimately results in defects in multiple physiological processes including the ability of the body to obtain energy from compounds such as carbohydrates or fats. Liver is the main iron storage organ and the multi-subunit protein ferritin stores iron. Much remains to be learned concerning how physiological changes in iron intake are transmitted into alterations in liver and whole body iron metabolism. Iron Regulatory Proteins (IRPs) are key regulators of mammalian iron metabolism because they modulate the synthesis of proteins that function in the uptake (transferrin receptor) and storage (ferritin) of iron. IRPs may also regulate synthesis of the enzyme mitochondrial aconitase (m-Acon). m-Acon is a component of tricarboxylic acid (TCA) cycle, one of the central energy producing pathways of the body. Since m-Acon converts citrate to isocitrate it appears that IRPs may modulate cellular metabolism of citrate. Several studies suggest that citrate may have an important role in iron trafficking within and between various tissues. Our objectives are to understand how changes in the iron content of the diet influence IRP function in liver. We will examine the temporal nature of the effect of iron intake on two targets of IRP action, ferritin and m-Acon. Finally, we will determine the molecular mechanism by which m-Acon synthesis is regulated by iron as well as examine the effects of altered m-Acon abundance on liver cell energy production.

ENSURING FOOD SAFETY

Panel Manager -Dr. Susan F. Barefoot, Clemson University
Program Director - Dr. Kathleen Ellwood

Safety of food products is of paramount importance to the producer, processor, distributor, and consumer. The research program in food safety focuses on research questions involving disease-causing microorganisms, their products, naturally occurring toxicants or drug residues which contaminate food. Projects may focus either on pre- or post-harvest/slaughter origin of the microbial agent or residue.

9702329 The Role of Acid Resistance in *Escherichia coli* 0157:H7 Colonization and Disease
Foster, J.W. **Grant 97-35201-4751**

University of South Alabama
Department of Microbiology and Immunology **$227,000**
Mobile, AL 36688 **3 Years**

Enterohemorrhagic *E. coli* (EHEC) cause a variety of dangerous gastrointestinal infections. EHEC Serotype 0157:H7 has recently emerged as an important foodborne pathogen that threatens many aspects of the food industry. A crucial feature of 0157:H7 pathogenesis is its ability to withstand stomach acidity. Our laboratory has identified three systems of acid resistance present in all *E. coli* and a fourth system dedicated to 0157:H7. Each system will protect cells to pH < 2.5 for several hours. We hypothesize that these acid resistance systems are induced during growth within livestock intestines and will persist over long periods of cold storage. One or more of these systems must contribute to the low infectious dose of 0157:H7 by protecting *E. coli* from gastric acidity and intestinal weak acids. Our **long terms goals** are to develop new strategies that will diminish the infectious character of this pathogens through an understanding of the molecular basis of acid resistance in *E. coli* with emphasis on the superior acid resistance of 0157:H7.

9702161 Quantitative Viability Assays for C*ryptosporidium parvum* and *Giardia lamblia*
Cliver, D.O. **Grant 97-35201-4607**

University of California, Davis
Department of Population Health and Reproduction **$44,000**
Davis, CA 95616 **1 Year**

Cryptosporidium parvum and *Giardia lamblia* are common waterborne agents whose potential for transmission via foods is increasingly being recognized. The objectives of this study are to develop quantitative viability assays for *C. parvum* and *G. lamblia* based on cell culture or *in vitro* culture and ELISA, and to evaluate the methods in trials of killing the protozoan oöcysts or cysts by various means pertinent to food safety. Oöcysts or cysts, respectively, will be inoculated into foods at risk of protozoan contamination (e.g., apple juice, shellfish, etc.); foods will be suspended in diluent as necessary, and the oöcysts or cysts will be recovered by immunomagnetic capture. The oöcysts or cysts will be treated to induce excystation, diluted serially, and inoculated into ELISA plate wells. Amplification of viable infectious agents will take place in the plate wells, during approximately 24 h at 37° C. Oöcysts of *C. parvum* will be amplified in plate wells that contain monolayers of BSC-1 cells; whereas *G. Iamblia* cysts will probably be amplified in artificial medium in the wells. Homologous antibody will be added and labeled indirectly with horseradish peroxidase. The wells will be washed, a color reaction carried out, and results determined in a standard ELISA plate reader. Control inocula will include oöcysts or cysts that have been inactivated by ultraviolet, formaldehyde, freezing, or heat. The tests will then be applied in inactivation trials with viable oöcysts or cysts in foods of interest or in water that might be used in food processing.

9702198 Molecular Stress Physiology of *Listeria monocytogenes*
Wilkinson, B.J.; Morse II, P.D. **Grant 97-35201-4478**

Illinois State University
Department of Biological Sciences **$113,000**
Normal, IL 61790-4120 **2 Years**

The bacterium *Listeria monocytogenes* is the causative agent of the foodborne disease listeriosis. The fatality rate of listeriosis is high and it is believed to be the leading cause of death from foodborne infections in the United States. Although *Listeria* can grow at the body temperature of an infected person, it has the unusual ability to also grow at refrigeration temperatures. Refrigeration can then in effect increase the *Listeria* content of a food. We are interested in the underlying mechanisms involved that permit the bacterium to grow at low temperatures. We will attempt to identify novel genes and proteins

involved in growth at low temperatures. A fatty acid known as anteiso branched chain fatty acid appears to play a critical role in growth at low temperatures. We will investigate the role of this fatty acid in maintaining membrane lipid fluidity at refrigeration temperatures. During food processing bacteria can become injured and undetectable by conventional culturing methods, but not be dead. We suspect that injured bacteria contain denatured proteins that must be degraded before the bacteria can grow, and we will investigate this hypothesis. It is hoped that these studies will provide the scientific basis that will lead to novel methods of control of *Listeria* and improved methods of detection of the organism.

9702560 Detoxification of Fumonisin by a Simple Fructose Reaction in Corn for Food
Hendrich, S.; Murphy, P.A.; Osweiler, G.O. **Grant 97-35201-4854**

Iowa State University
Department of Food Science and Human Nutrition **$110,000**
Ames, IA 5001l **2 Years**

The toxin, fumonisin B1, is found in corn everywhere. A suspected cancer-causing agent in humans, fumonisin B l requires its amine group, a simple nitrogen-containing portion of the molecule, for its toxic action. Reacting this amine with simple sugars, such as fructose, is likely to block fumonisin toxicity, as we have previously demonstrated in a one-month model of liver cancer development in rats. Our objectives are: 1) To determine the toxicity of fructose-FBl products in: a) a study of short-term toxic effects in pigs and b) a field-test feeding rats a fumonisin contaminated corn food that we have attempted to detoxify; and 2) To determine the processing conditions for the reactions of fructose and glucose with fumonisin to occur in corn-based foods. Objective l will be accomplished by feeding studies in pigs (for 2 weeks) and rats (for 4 months), comparing toxicity of pure fumonisin B 1 with a fumonisin B1-fructose product and with corn contaminated with fumonisin and corn reacted to detoxify the fumonisin. Toxicity will be assessed by blood chemical changes and microscopic examination of tissues from the test animals. Objective 2 will determine the feasibility of performing this detoxification reaction in human foods, characterizing FB-reducing sugar reactions and the nature of the detoxification product(s), using a variety of chemical analytical techniques. These studies may provide a practical approach to the problem of natural toxins, increasing the safety of the food supply by detoxifying a natural toxin that occurs in com everywhere.

9702755 *Salmonella enteritidis* Heterophil Resistance
Kramer, T.T.; Minion, F.C.; Trampel, D.W. **Grant 97-35201-4608**

Iowa State University
Veterinary Medical Research Institute **$164,000**
Ames, IA 50011 **3 Years**

Egg-transmitted human salmonellosis is the most widespread food safety problem in the developed world. Over the past two decades, the number of *Salmonella* infections has increased dramatically in the United States, mostly due to *Salmonella enteritidis* var. *enteritidis* (SE) infections of eggs and egg products. The difficulty in controlling SE is primarily due to the low, but significant incidence of infected eggs. We have isolated two less virulent SE mutants which are only briefly shed by infected birds, are effective in protecting birds against virulent challenge, and prevent egg transmission of virulent SE. We propose to utilize these mutants to study the molecular basis of SE virulence and to identify genes involved in immune cell resistance so that safer vaccines can be developed. The following specific aims will be addressed: 1) We will complete the assessment of the SE mutants in chickens in order to assess their pattern of colonization of infected tissues and affinity for egg laying tissues; 2) The genes involved in immune cell resistance will be identified by screening complemented strains in cell cultures; 3) Mutations will be constructed in these genes in the wild type strain in order to confirm their role in immune cell resistance; and 4) The mutants will be assessed for virulence and egg transmissibility in chickens. For unknown reasons, immune cell resistance is directly linked to lowered virulence in SE. These studies will shed light on the possible mechanisms involved and add to our understanding of *Salmonella* pathogenesis

9702553 Extracellular Sporulation Signals of *Clostridium perfringens*
Labbe, R. **Grant 97-35201-4507**

University of Massachusetts
Department of Food Science **$148,000**
Amherst, MA 01003-1410 **3 Years**

Clostridium perfringens has established itself as a leading cause of human foodborne illness in the U.S. This organism produces heat resistant spores. An enterotoxin is produced by some strains during sporulation and therefore the sequence of events leading to spore formation are especially important. Yet virtually nothing is know about the early events of this process. We have identified a sporulation factor (*C. perfringens* sporulation fact [CPSF]) produced by both enterotoxin-positive and -negative strains which stimulate the onset of sporulation and enterotoxin formation by this organism. The product(s) may be part

of a signal transduction system. The signal transduction system in bacteria monitors the bacteria's environment and reacts to changes by chemical signals to the interior of cell. We will develop conditions to optimize the levels of this product then attempt to isolate and characterize it.

Raw protein foods are commonly contaminated with both enterotoxin-positive and enterotoxin-negative strains and the ability of enterotoxin-negative strains to stimulate sporulation and enterotoxin formation of co-cultured enterotoxin-positive strains will be determined in laboratory media and in a model food system. Such an ability by enterotoxin negative strains could contribute to periodically-reported *C. perfringens* outbreaks having short incubation periods and may also identify a role for enterotoxin-negative strains in promoting sporulation and enterotoxin formation in the human intestine following ingestion of temperature-abused foods
containing high levels of vegetative cells of both toxin types.

9702070 Enhanced Green Fluorescent Protein Expression in *Escherichia coli* to Study Adherence to Meat
McLandsborough, L.A. **Grant 97-35201-4508**

University of Massachusetts
Department of Food Science
Amherst, MA 01003
$92,000
2 Years

The United States Department of Agriculture Food Safety Inspection Service (USDA/FSIS) recently enacted a regulation that requires that all meat and poultry processing plants develop a hazard analysis critical control points (HACCP) program. The aim of this regulation is reduce the presence of infectious bacteria on the surface of meats, ground beef, and poultry products. There has been much research focusing upon methods for meat disinfection, even though there is little known about how bacterial stick to meat surfaces. This proposal will develop a microscopic experimental system that will investigate bacterial adhesion to meat surfaces. Knowledge of the interaction between bacteria and meat surfaces will lead to improved methods of detection and meat decontamination.

This project will create *E. coli* strains that express enhanced fluorescent green protein (EGFP) and use these constructs to study bacterial adhesion and growth on meat surfaces by laser scanning confocal microscopy (LSCM). Both non-pathogenic *E. coli* and pathogenic strains will be constructed. This model system will allow experiments to be designed to determine the specificity of the adhesion and for analysis of the distribution of bacteria to meat structures. The scientific significance of this study is the novel system for investigation into the specific nature and parameters involved in bacterial adhesion to meat at a cellular level. The practical applications of this study will be the generation of basic knowledge that can be applied to evaluation of differential binding of pathogens (and indicator organisms) and the application of this knowledge to the wash steps during meat processing.

9702102 Detection and Analysis of *Staphylococcus aureus* Enterotoxin A in Food
Rasooly, L.; Rasooly, A. **Grant 97-35201-4569**

Johns Hopkins University
Department of Microbiology and Molecular Immunology
Baltimore, MD 21205
$133,000
2 Years

The goal of this project is to increase food safety by developing the next generation of detection and analysis methodology for bacterial toxins in food, using *Staphylococcus aureus* enterotoxin A (SEA) as a model. The proposal aims to develop two technologies: a cell culture based assay of SEA activity and biosensor methodology for immediate automated detection of SEA in food. The two different approaches will complement each other since they address two sides of the same problem. Biosensor detection allows rapid detection of the toxin in food, while the cell culture methodology supplies the information on biological activity of the toxin. These two methods are expected to overcome the limitations of current immunological and animal-based tests for toxins in food. The cell culture based activity assay will be developed by exploiting the toxin's ability to stimulate division of lymphocytes. Biosensor technology represents a new approach to food safety analysis: real-time analysis. Biosensors can translate biological measurements into electronic signals enabling immediate analysis and automation. A novel methodology applying biosensor technology to food testing is proposed here. The aim of this project is to develop new testing methodologies which will aid food production and food regulation, and may increase food safety and quality.

9702074 Intimin: Candidate for an *Escherichia coli* 0157:H7 Anti-Transmission Vaccine
O'Brien, A.D.; Nystrom, E.A.; Stewart, C.N. **Grant 97-35201-4578**

Uniformed Services University of the Health Sciences
Department of Microbiology and Immunology **$232,456**
Bethesda, MD 20814-4799 **3 Years**

Enterohemorrhagic *Escherichia coli* (EHEC) 0157:H7 is the most common infectious cause of bloody diarrhea in the U.S., and an occasional consequence of this infection, the hemolytic uremic syndrome, is the primary cause of acute kidney failure in U.S. children. Most U.S. cases of EHEC 0157:H7 disease have occurred after ingestion of under cooked, contaminated hamburger. Cattle are reported to be asymptomatically and sporadically infected with this organism. EHEC have been shown to adhere to the intestinal epithelium of neonatal calves via a bacterial surface protein called intimin. The long-term goal of our project is to develop an inexpensive vaccine to prevent cattle from becoming infected with EHEC and, thus, prevent transmission from cattle to humans. To achieve this objective, we will: i) evaluate whether intimin is required for EHEC 0157:H7 colonization of older calves; ii) assess whether oral administration of anti-intimin antibodies interferes with intestinal colonization and lesion formation caused by EHEC 0157:H7 in piglets, a surrogate for calves; iii) test whether pregnant pigs administered intimin by a non-oral route elicit anti-intimin antibody responses in serum, colostrum, and milk and whether suckling piglets born of these immunized sows are protected from infection with *E. coli* 0157:H7; iv) compare the antibody responses of mice to intimin and a set of intimin fragments administered by different routes and identify the smallest fragment that elicits antibodies capable of blocking EHEC adherence to epithelial cells; and v) develop a plant that expresses intimin or a fragment thereof as a potential edible vaccine for cattle.

9703022 Survival and Virulence of Enterohemorrhagic *Escherichia coli* (EHEC) as Affected by pH and Water Activity
Meng, J. **Grant 97-35201-4904**

University of Maryland **New Investigator Award**
Department of Nutrition and Food Science **$87,000**
College Park, MD 20742 **2 Years**

Enterohemorrhagic *Escherichia coli* (EHEC) have caused a series of foodborne outbreaks of bloody diarrhea as well as serious complications, including hemolytic uremic syndrome (HUS). While research efforts have been focused on *E. coli* 0157:H7, it is becoming more evident that other serotypes of EHEC can also be associated with human diseases. An increasing number of non-0157 EHEC have been isolated from humans suffering from HUS and diarrhea. A variety of foods have been implicated in *E. col i* 0157:H7 outbreaks, particularly foods of bovine origin. Certain foods such as apple cider and dry-cured salami that were considered safe and ready to eat, and are generally not heated before consumption have been identified as transmitting vehicle in *E. coli* 0157:H7 outbreaks. Unlike 0157:H7, most of non-0157 EHEC serotypes have been isolated from sporadic cases, hence, the significance of food as vehicle for transmitting non-0157 EHEC is not clear. It has been shown that bacterial regulatory responses to environmental conditions are tied to virulence gene expression and that stressful signals in a hostile environment (e.g. acidic and/or dry conditions) can be utilized to induce/enhance virulence gene expression by pathogenic microorganisms. Foodborne pathogens having been exposed to such conditions may become more virulent. We propose to study: 1) Survival of EHEC strains (mainly non-0157:H7) as affected by pH and water activity; and 2) Virulence of EHEC strains as affected by pH and water activity.

9702069 Symposia on Microbial Food Borne Hazards - Basic Research/Industry/Regulatory Concerns
Bunning, V.K. **Grant 97-35201-4504**

DHHS Food and Drug Administration
Immunology Branch **$6,000**
Laurel, MD 20708-2476 **1 Year**

The Food Microbiology Research Conference (FMRC) focuses on the presentation of basic/applied research by scientists within academia, government, and industry. The activities of the FMRC are governed by a set of bylaws, which were adapted as part of the process of gaining tax exempt status (private/nonprofit), thereby providing formal structure to the conference's financial management. FMRC meets every two years in the Chicago area, participation is by invitation, and the program format (panel discussion; individual seminars; symposia) is designed by an Executive Committee. The goal of the Conference is to advance knowledge and understanding in the area of food microbiology. FMRC meeting represent one of the few regularly held gatherings exclusively devoted to food microbiology. Industry/regulatory concerns are incorporated into the program for timely and relevant research topics. The XVI FMRC is scheduled for 9-12 November 1997 at the Ramada Inn, Chicago-O'Hare. Confirmed symposia include: Molecular Approaches for Food Safety Assurance; Resistance-Control-Host Response to Bacterial Pathogens; Developments in Bacterial Inactivation and Reduced Consumer Risk; Roundtable panel on Zero Tolerance/Risk; and

General Topics. Invited speakers and chosen symposia topics are designed to promote research/industry/regulatory interaction, thereby furthering the overall goal of enhancing food safety.

9702545 Recombinant Antibodies to Natural Toxicants
Pestka, J.J.; Linz, J.E.; Hart, L.P. **Grant 97-35201-4579**

Michigan State University
Department of Food Science and Human Nutrition **$116,000**
East Lansing, Ml 48824-1224 **2 Years**

There has been increased use by government agencies and the food industry of rapid antibody-based immunoassay in a first-tier screen for harmful toxins and microbial pathogens in foods. The antibodies used in these assays have been developed in animals such as rabbits or in tissue culture systems. Using recombinant DNA technology, it is now possible to engineer specific antibody reagents for improved food safety screening. The immediate advantages of recombinant antibodies are threefold. First, these antibodies can be genetically manipulated to improve sensitivity and greatly reduce assay time. Antibodies can also be designed that have specificity for groups or broad classes of toxicants or harmful microbes. Second, this approach will diminish the use of animals and animal products (eg. fetal calf serum) for antibody production. Third, since recombinant antibodies will be produced in bacteria, the cost of the basic reagent will be as much as 10-fold less than that for animal or tissue culture systems. Thus, recombinant antibodies could be immediately useful in enhancing existing and new assays for toxins and microbes in foods.

This proposal seeks to genetically engineer novel antibodies to an important group of natural toxins known as the *Fusarium* mycotoxins which commonly contaminate wheat, corn, rice and barley. Specifically, antibodies to fumonisin, vomitoxin and zearalenone will be prepared in bacteria and then these antibodies will be applied to testing for these harmful toxicants in food. From the perspective of food safety, the general approaches developed in this research will be amenable to improved detection of natural toxicants, chemical contaminants as well as bacterial pathogens and their toxins. Over the long term, cloned antibody sequences may find novel uses such as (1) immunization of food producing animals prevent toxic residues or pathogens in meats and poultry, (2) development of low cost procedures for removing toxicants from milk and dairy foods, and (3) expression in plants to neutralize toxicity.

9702552 Adhesins for Colonization of Chickens & Their Use in Preventive of Salmonellosis
Curtiss, R. III; Wilmes-Riesenberg, M.R. **Grant 97-35201-4936**

Washington University
Department of Biology **$156,000**
St. Louis, MO 63130-4899 **3 Years**

The incidence of infection resulting from food borne pathogens continue to increase worldwide despite extensive research and changes at the production and processing levels. A 1996 CDC study indicated that *Salmonella* accounted for the majority of the bacterial food borne disease outbreaks from 1988 to 1992. Our long-term objective is to reduce or eliminate *Salmonella* colonization of poultry, which would in turn, result in a reduction in the shedding of *Salmonella* in feces, its transmission to eggs, and the cross-contamination which occurs during processing. An understanding of the mechanism of *Salmonella* adherence to chicken cells could be particularly valuable when developing strategies to eliminate *Salmonella* contamination of poultry. Our preliminary data support the hypothesis that the *Salmonella* bacterium expresses gene(s) encoding an "adhesin" protein in response to high iron concentrations, and this adhesin is involved in binding the bacterial pathogen to a host cell. The goals of this proposal are (1) to identify the gene(s) encoding the iron-induced adhesin from *Salmonella typhimurium*, (2) to evaluate the role of the iron-induced adhesin in the adherence of the *Salmonella* to avian cells and (3) to determine if the iron-induced adhesin is made by other *Salmonella* species which colonize chickens. We will identify mutants unable to synthesize this adhesin and these will be evaluated using tissue culture and animal models. Ultimately, this information will be used to design methods to eliminate *Salmonella* in poultry either by contributing to the development of a live oral vaccine, or by identifying possible changes in the slaughtering procedure to reduce *Salmonella* cross-contamination.

9702551 Incidence and Fate of Moniliformin in Corn and Heat Processed Corn Products
Bullerman, L.B. **Grant 97-35201-2343**

University of Nebraska
Department of Food Science and Technology **$97,000**
Lincoln, NE 68583-0919 **2 Years**

Moniliformin is a highly toxic substance produced by *Fusarium proliferatum* and *Fusarium sublutinans*, molds commonly found on corn. Moniliformin has also been found in corn from different parts of the world, though the incidence and levels in corn and corn-based food products in the U.S. are not well documented. Considering the toxicity of moniliformin and the potential risk of chronic long-term consumption of it in corn-based foods, it is very important to know the extent of contamination

and heat stability of moniliformin in corn and corn-based foods. The overall objective is to determine the incidence and levels of moniliformin in U.S. corn and corn-based foods, and the effects of heat, as applied in basic thermal processing of corn, on the stability of moniliformin. Specific objectives are to determine: 1) the incidence and amounts of moniliformin in U.S. corn and corn-based foods; 2) the effect of heat on the stability of pure moniliformin in water at different temperatures, pH levels and heating times; and 3) the effect of selected thermal processes, including extrusion, alkaline processing (tortilla process) and baking on the stability of moniliformin in corn. To accomplish the objectives, corn and corn-based foods will be obtained from commercial food channels throughout the U.S. and analyzed for the presence and amounts of moniliformin. Heat stability of moniliformin in both water and corn substrates will also be studied. After heating in water or by the selected process, the presence and amount of moniliformin remaining will be determined by high performance liquid chromatography (HPLC).

9702494 Modeling the Interactions of Pathogenic and Biocontrol Bacteria for Applications in Foods
Breidt, F.; Fleming, H.P. **Grant 97-35201-4506**

USDA Agricultural Research Service
Food Science Research Unit **$86,000**
Raleigh, NC 27695-7624 **2 Years**

The objective of this research is to develop a safe method for preventing the growth of pathogenic bacteria in minimally processed, refrigerated foods. A biocontrol strategy will be used which involves bacterial competition to accomplish this task. Lactic acid bacteria which are commonly used in various food fermentations (dairy, meat, vegetables) will be added as biocontrol agents to prevent the growth of pathogenic bacteria in minimally processed foods. If a food protected by this type of biocontrol strategy should spoil due to improper refrigeration or other reasons, the lactic acid bacterium should grow and competitively prevent the growth of potentially harmful bacteria. Although the food may not taste good because of the acid produced, the product would not be unsafe. We have developed a mathematical model that predicts the outcome of the competitive growth of bacteria. The model may be useful in determining which lactic acid bacteria should be chosen as biocontrol agents, and how the growth of selected lactic acid bacteria will affect the growth of pathogenic or disease-causing bacteria. Our research will involve growing both biocontrol and pathogenic bacteria, singly and in mixed culture, in vegetable broth and minimally processed vegetable products. Using the model to help interpret the data from these experiments, we hope to gain insights into which factors such as growth rates, production of inhibitory compounds, or sensitivity of the cells to these inhibitors are most important to the predominance of one bacterial culture over another. While we will primarily investigate biocontrol applications for refrigerated vegetable products, it is hoped that the principles learned in these studies can be applied to biocontrol applications for a variety of foods.

9702192 *Salmonella* in Modern Swine Production Systems. Risk Factors for Fecal Shedding by Finished Pigs
Davies, P.R. **Grant 97-35201-4479**

North Carolina State University
College of Veterinary Medicine **$241,000**
Raleigh, NC 27606-8401 **3 Years**

Control of Food borne disease is best achieved through appropriate actions in all sectors of the farm to table continuum. Salmonellosis is a major foodborne disease worldwide and *Salmonella* is the foodborne pathogen of greatest importance in modern swine production. Systems for producing swine have changed radically in recent years, in association with increases in average herd size. Knowledge of the epidemiology of *Salmonella* infections in modern swine production systems is minimal, but is necessary to identify appropriate measures to reduce the risk of foodborne disease to people and ensure access to international markets. Specific objectives of this project are to determine: 1) risk factors for *Salmonella* prevalence in finishing pigs raised on slotted concrete floors in barns managed all-in/all-out, within multiple-site production systems; and 2) the relative importance of *Salmonella* infection in nurseries or the finishing environment as determinants of *Salmonella* infection in finishing hogs. Prevalence and serotypes of *Salmonella* will be determined by fecal cultures in finishing pigs, raised at specialist finishing sites. The sites chosen will be typical of modern systems that are predominantly, and increasingly, used for pork production in the USA. Feed and environmental samples will also be cultured. Data on management and environmental factors will be collected and examined for associations with *Salmonella* prevalence. The information obtained will be relevant to a large and increasing segment of the national swine industry and will aid in defining the most efficient options for reducing *Salmonella* in the pork supply.

9702162 Experimental Campylobacter Vaccine
Nachamkin, I. **Grant 97-35201-1980**

University of Pennsylvania
Department of Pathology and Laboratory Medicine **$138,000**
Philadelphia, PA 19104-4283 **2 Years**

Campylobacter jejuni is a major cause of gastrointestinal infection and man and is the most common cause of sporadic diarrheal illness in the U.S. *Campylobacter* infection is primarily a foodborne disease with poultry being the single most important vehicle for transmitting the disease. A number of immunological approaches to reducing or eliminating *Campylobacter* from poultry are currently being investigated including the use of vaccines. The mechanism by which *Campylobacter* colonizes the chick GI tract is not completely understood but flagella are important colonization factors. We expressed the full length *Campylobacter* flagellin gene, flaA, in an avirulent *Salmonella typhimurium* vaccine vector and tested several vaccine constructs in 4 day old chicks for immunogenicity and protection. During the past funding period, we showed that these vaccines were highly immunogenic and induced anti-flagellin antibodies using a two-dose regimen. When animals were challenged 3 weeks after vaccination with the homologous strain of *C. jejuni*, vaccines conferred >95% homologous protection against cecal colonication. In the next funding period, we will extend these studies to 1) assess the ability of these vaccines to confer cross-protection with different flaA types of *C. jejuni*, 2) determine the minimal amount of time needed post-immunization to confer protective immunity, 3) determine the minimal *C. jejuni* challenge dose in which complete protection occurs, 4) determine whether the bivalent vaccine confers protection against *Salmonella* infection and 5) determine the smallest flagellin fragment that can elicit protective immunity. An immunogenic, broadly cross-reactive vaccine should be useful in improving the safety of poultry for human consumption.

9702108 Food Pathogen Biosensors for Rapid Safety Measurements of Meat
Rand, A.G.; Letcher, S.V.; Brown, C.W.; Sperry, J.F. **Grant 97-35201-4480**

University of Rhode Island
Fiber Optic and Biosensor Research Group **$96,205**
Kingston, RI 02881 **2 Years**

Classical procedures for the detection of microbial pathogens in meats are slow and labor intensive. Rapid methods currently available are either complex, require potentially hazardous and expensive materials, or utilize a pre-enrichment step of 18-24 hours to grow up enough cells for detection. This project will establish that biosensors employing immobilized antibodies specific for meat pathogens can be successfully utilized for biomonitoring of contamination in food products. One approach will utilize fiber optics to analyze the optical excitation and emission properties of immobilized antibodies and attached pathogens on the surfaces of gold coated silicon. The second biosensor will continue to explore the potential of the Quartz Crystal Microbalance with reusable piezoelectric quartz crystals containing attached antibodies. The maximum response of these biosensors for determination of microbial cell concentrations of pathogens in meat products will be established. Consumer demand for fresh, less processed food, such as meat, makes the need to ensure microbial safety of products very clear. This project provides the opportunity for a multidisciplinary effort to create specific biosensors for rapid and early detection of pathogen contamination in meat. These devices have the potential for specifically selecting food pathogens from among the total microbial load within minutes and measuring the concentration as real-time analysis on site. The capability for miniaturization and portability emphasizes the possibilities that this new technology will provide the tools for effective monitoring programs. The ability for rapid early detection of pathogens will enhance the safety and quality of U.S. meat products.

9702568 *Salmonella typhimurium* Genes Required for Systemic Infection of Cattle
Tsolis, R.M. **Grant 97-35201-4505**

Texas A&M University **Postdoctoral Fellowship**
Department of Veterinary Pathobiology **$90,000**
College Station, TX 77843-4467 **2 Years**

Salmonellosis is the most frequent food-borne illness in the U.S. and is usually contracted by consumption of meat and dairy products from infected livestock. Little is known about genes allowing *Salmonella typhimurium* to cause systemic infection in cattle, an important meat source in the U.S. Since systemic infection can lead to a chronic carrier state, information about the mechanisms used by *S. typhimurium* to establish systemic infection is relevant to development of strategies to eliminate this pathogen from cattle. The goals of this project are the identification and characterization of bacterial genes which enable *S. typhimurium* to cause systemic infection in cattle. The role that these genes play during infection will be examined by determining the ability of attenuated bacterial mutants to spread to different organs in cattle. Finally, by determining whether the same set of virulence genes identified in cattle is also required for infection of the mouse, we will determine whether any of the genes identified in this study are host-specific adaptations to causing disease in cattle. The results of this research will help

to develop strategies for reducing the number of carrier animals from cattle herds as well as for the detection of *Salmonella* in meat and dairy products, thereby increasing food safety.

9702558 Fumonisins: Immunology, Genetics and Enzymology
Chu, F.S.; Leslie, J.F. **Grant 97-35201-4680**

University of Wisconsin, Madison
Department of Food Microbiology & Toxicology
Madison, WI 53706
$129,897
2 Years

Fumonisins (Fms) are a group of mycotoxins produced primarily by the fungus *Fusarium moniliforme*, FmB1, the major mycotoxin in this group, is a weak carcinogen and induces apoptosis both in animals and plants. It also is responsible for leukoencephalomalacia in horses and for swine edema syndrome/swine mystery disease. Because of the widespread occurrence of this group of mycotoxins in corn and related foods and their carcinogenicity and potent cancer promoting activity, this group of mycotoxins is potentially hazardous to human and animal health. Using mutant cultures and a combination of immunochemical and chemical methods, we plan to identify the major steps, intermediates and enzymes involved in the biosynthesis of Fm. The methodology developed in the proposed work could be used for further studies of the conditions conducive to the formation of Fms in the field and during storage. Different tools and mutants developed from the present study will be shared with other scientists for related studies. This study is a critical step in the development of methods to control Fm formation.